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Elastic fiber proteins in the glomerular mesangium in vivo and in cell culture

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Background. Glomerular capillaries of the mammalian kidney are exposed to high intraluminal hydrostatic pressures and require elastic constraint to maintain size, shape, and integrity. Previous morphological and functional studies indicated that the extracellular matrices of glomeruli, that is, basement membrane and mesangial matrix, contribute to glomerular resilience and mechanical stability. Immunofluorescence microscopy findings demonstrated elastic fiber components to be located in the renal vasculature, including glomeruli. The aim of this study was to clarify the exact glomerular localization, composition, and cellular production of these proteins.

Methods. We examined the renal distribution of the elastic fiber proteins fibrillin-1, emilin, microfibril-associated glycoproteins (MAGPs) 1 and 2, latent transforming growth factor-binding protein-1 (LTBP-1), and elastin using immunohistology and immunoelectron microscopy of human, rat, and mouse kidneys. In mesangial cell cultures, we also studied the expression and extracellular deposition of such proteins by use of Northern blotting and immunocytochemistry.

Results. Fibrillin-1, emilin, MAGPs 1 and 2, and LTBP-1 were present in glomeruli of mouse, rat, and human kidney, where they were located predominantly in the mesangial extracellular matrix underlying glomerular endothelium and basement membrane. Several of these proteins, as well as elastin, were also expressed in the renal vasculature. While elastin localized to the glomerular vascular pole in afferent and efferent arterioles extending to Bowman's capsule, it was not found in the glomerular capillary tuft. Cultured mesangial cells of rat, mouse, and human kidneys expressed mRNAs of fibrillin-1, emilin, MAGP-2, and elastin, and the respective proteins localized within and outside of mesangial cells, as shown by immunocytochemistry. mRNA expression of fibrillin-1, emilin, and elastin was strong in quiescent mesangial cells; their gene expression was further up-regulated by transforming growth factor-β1, while it was transiently reduced when cells were exposed to mitogenic 10% fetal calf serum and platelet-derived growth factor.

Conclusions. These findings demonstrate that specific elastic fiber proteins are produced and secreted by mesangial cells. This process is regulated by growth factors. Their abundance in the extracellular matrix of the mesangium is in keeping with the concept that elastic fiber proteins contribute to the mechanical stability and elastic strength of the glomerular capillary tuft.

Key words: hydrostatic pressure, cell elasticity, renal vasculature, extracellular matrix, growth factor, glomerular capillary tuft, transforming growth factor-β.

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The glomerular capillary tufts of adult mammals are exposed to high and variable intraluminal hydrostatic pressures and require elastic strength to maintain capillary size, shape, and integrity [1–6]. Several studies have examined what structures may contribute to the resilience of the glomerular capillaries and provide mechanical properties that are essential for the maintenance of glomeruli [1–3, 7]. Based on morphological analyses, it has been proposed that the contractile mesangial cells (MCs) [1, 2] as well as podocytes [1, 8] generate inwardly directed forces that afford stability to the capillary loop by opposing the expansile force of high hydrostatic glomerular capillary pressure. Cortes et al have investigated the distensibility of isolated rat, rabbit, and human glomeruli, which were perfused at different pressure levels [3]. They found that the MC tone (for example, in response to angiotensin II) contributes little to the elastic constraint and recoil of glomeruli. Rather, these authors observed that the major determinants affecting glomerular volume and capillary diameter are the glomerular capillary wall tension (depending on prevailing hydrostatic pressure and basal capillary width) and elastic properties of the glomerular extracellular matrix (ECM), that is, the glomerular basement membrane (GBM) and mesangial matrix

[3, 9]. Based on these experimental results, it was concluded that composition and distribution of the ECM are the most important determinants of the glomerular mechanical properties.

Ultrastructural studies by Kriz et al emphasized the existence of a network of fine electron-dense fibrillar structures in the mesangial ECM [1, 2, 7, 10], which had been originally described by Farquhar and Palade in 1962 [11] and later by Reale et al [12]. These filaments are no thicker than 10 to 15 nm and often appear to be arranged in bundles [1, 2, 13]. In the glomerulus, they are located preferentially in subendothelial location, particularly at so-called mesangial angles found at the waist of glomerular capillaries, that is, where the endothelial–mesangial interface meets the GBM [1, 2]. It has been suggested that these fibrillar structures may serve anchoring as well as elastic functions in the glomerular capillary tuft [1, 10]. However, their composition and regulation, as well as the nature of their interactions with mesangial and endothelial cells and with the GBM are presently uncertain.

By electron microscopy, the filamentous structures in the mesangial ECM resemble microfibrils that have been identified in elastic as well as nonelastic connective tissues, for example, of the vasculature, skin, tendons, and the eye [13–15]. Microfibrils are extensible linear polymers. In elastic fibers, microfibrils are associated with elastin, but they can also be found in elastin-free bundles [13, 15, 16]. The main constituents of microfibrils are fibrillins 1 and 2 [16–18]. Other glycoproteins related to elastic fibers and microfibrils include emilin [13, 19, 20] and microfibril-associated glycoproteins (MAGPs) 1 and 2 [21, 22]. Immunohistological surveys of the organ distribution of such proteins were performed in chick, rat, mouse, bovine, and human tissues and revealed their presence in many organs, including the kidney, where the vasculature, glomeruli, and/or the peritubular connective tissue stained positively for the respective proteins [20, 21, 23, 24]. While the immunofluorescence staining pattern observed in glomeruli appeared to reflect mesangial deposition of the employed antibodies, no information was obtained with regard to the precise glomerular localization and origin of production of the examined proteins. Also, it remains unclear what factors regulate their expression and abundance in the glomerulus.

To clarify the renal and glomerular localization of the elastic fiber proteins fibrillin-1, emilin as well as elastin, we examined renal tissue of normal adult human, rat, and mouse using immunohistology and immunoelectron microscopy. These findings were compared with the immunolocalization of other proteins that have been described to be components of or interact with microfibrillar structures, such as MAGPs 1 and 2 and latent transforming growth factor-binding protein-1 (LTBP-1) [25–28]. Since our initial observations in rat kidney sections had confirmed that microfibrillar components, indeed, are present in the mesangial ECM, we also examined the expression of several proteins, that is, fibrillin-1, emilin, MAGPs 1 and 2, LTBP-1, as well as elastin by cultured rat, mouse, and human MCs and performed experiments to study the regulation of their expression at the mRNA level. The results of these immunolocalization studies and MC culture experiments demonstrate that multiple elastic fiber proteins are expressed and produced by MCs and are organized in the mesangial ECM. Our findings are in keeping with the concept that elastic fiber proteins contribute to stability and elastic constraint of the glomerular capillary ultrafiltration apparatus. These new observations should stimulate further experiments to elucidate expression and functions of elastic fiber proteins in normal and diseased glomeruli.

METHODS

Immunohistochemistry

Normal kidney tissue was obtained from young adult male mice ($N = 4$, C57/black 6; Charles River, Sulzfeld, Germany), rats ($N = 4$, Sprague-Dawley; Charles River) and three human adult donor kidneys that were not used for transplantation (because of either laceration of a short renal artery or last-minute problems with graft recipient). Portions of decapsulated kidneys were immediately snap frozen in liquid nitrogen or were fixed in methyl-Carnoy solution (60% methanol, 30% chloroform, and 10% glacial acetic acid) or 4% paraformaldehyde. After overnight fixation in methyl-Carnoy solution or paraformaldehyde, tissues were dehydrated in increasing concentrations of methanol (or ethanol for paraformaldehyde-fixed tissue), followed by 100% isopropanol. After embedding in paraffin, 3 μm sections were cut with a Leitz SM 2000 R microtome (Leica Instruments, Nussloch, Germany). After deparaffinization, endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 20 minutes at room temperature. Sections were layered with the primary antibody and incubated at 4°C overnight. After the addition of the secondary antibody (dilution 1:500; biotin-conjugated, goat anti-rabbit or anti-mouse immunoglobulin G), the sections were incubated with avidin-conjugated horseradish peroxidase complex (Vectastain DAB kit; Vector Laboratories, Burlingame, CA, USA) and exposed to 0.1% diaminobenzidine tetrahydrochloride and 0.02% H_2O_2 as the peroxidase substrate. Each slide was counterstained with hematoxylin. As negative controls, equimolar concentrations of preimmune rabbit or mouse immunoglobulin G or an irrelevant secondary antibody were used.

For localization by immunofluorescence microscopy, 3 μm cryostat kidney sections were air dried for 10 minutes, fixed in cold acetone for 10 minutes, washed twice in Tris-buffered saline (TBS)/1% bovine serum albumin

(BSA), and incubated with 100% fetal calf serum (FCS) for 30 minutes at 37°C. Primary antibodies were applied overnight at 4°C. After washing, sections were incubated with secondary antibodies, CY2-labeled goat anti-rabbit or anti-mouse immunoglobulin G (Dianova, Hamburg, Germany) for two hours. Double immunofluorescence was performed by applying primary antibodies simultaneously overnight at 4°C. After washing, sections were incubated with secondary antibodies, CY2-labeled goat anti-mouse immunoglobulin G, and CY3-labeled goat anti-rabbit immunoglobulin G (both from Dianova) at the same time for two hours. Washed sections were then covered with Tris-buffered Mowiol, pH 8.6 (Hoechst, Frankfurt, Germany).

Immunoelectron microscopy

Postembedding immunogold labeling was performed as previously described [29]. Tissue specimens of normal mouse, rat, and human kidney were fixed in a freshly made solution of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4) for three hours at 4°C. After rinsing, specimens were dehydrated serially to 70% ethanol at −20°C, embedded in LRWhite resin, and polymerized for 24 hours at 45°C.

Ultrathin sections were incubated successively in drops of TBS, 0.05 mol/L glycine in TBS, 0.5% ovalbumin, and 0.5% fish gelatin in TBS, primary antibodies diluted in TBS-ovalbumin overnight at 4°C, and 10 nm gold-conjugated secondary antibody (BioCell, Cardiff, Wales, UK) diluted 1:30 in TBS-ovalbumin for one hour at room temperature. After rinsing, sections were stained with uranyl acetate and examined with an EM 906 electron microscope (Leo, Oberkochen, Germany). For certain antibodies (MAGPs 1 and 2 and LTBP-1), pretreatment of sections with 0.1% trypsin in TBS (pH 7.8) for 10 minutes at room temperature or microwave heating in 0.01 mol/L citrate buffer (pH 6.0) was required. For preembedding labeling, 50 μm thick cryostat sections of unfixed kidney tissue were blocked in 1% BSA in phosphate-buffered saline (PBS) for 30 minutes, incubated in primary antibody diluted in BSA-PBS overnight at 4°C, followed by the 10 nm gold-conjugated secondary antibody diluted 1:30 in BSA-PBS for one hour at room temperature. After washing, sections were fixed in 2.5% glutaraldehyde in PBS for one hour and then in 1% osmium tetroxide in PBS for one hour at 4°C. Sections were dehydrated in an ethanol series and embedded in epoxy resin.

To improve the electron microscopic demonstration of microfibrils in the mesangium, kidney tissue was fixed in 2.5% glutaraldehyde and 3% tannic acid in PBS overnight at 4°C, postfixed in 2% buffered osmium tetroxide, and then embedded in epoxy resin according to standard protocols. Ultrathin sections were stained with uranyl acetate and lead citrate.

Mesangial cell culture

Mesangial cells were isolated from normal mouse, rat, or human kidneys, as described previously [30]. Cultured MCs showed a typical vascular smooth muscle-like morphology and positive immunostaining for smooth muscle α-actin and myosin as well as α_8-integrin [31]. Rat MCs also stained positively for Thy 1.1 antigen (Biozol, Eching, Germany) [31]. MCs of mouse and rat were grown in Dulbecco's modified Eagle's medium (Life Technologies, Eggenstein, Germany) containing 10% FCS, 5 μg/mL insulin, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine (Sigma, Deisenhofen, Germany) in a 95% air/5% CO_2 humidified atmosphere at 37°C. Human MCs were grown in Waymouth medium containing 20% FCS, nonessential amino acids, 5 μg/mL insulin, 100 μ/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L L-glutamin, 1 mmol/L pyruvate, 10 mmol/L HEPES. MCs were used for experiments in passages 8 through 18 (mouse and rat) or 12 through 20 (human).

Immunocytochemistry of mesangial cell cultures

Mesangial cells of subconfluent cultures were harvested with trypsin-ethylenediaminetetraacetic acid (EDTA) and were washed and seeded (5000 cells/well) on glass eight-well chamber slides blocked with 2% BSA. Cells were allowed to adhere for 24 hours, followed by serum deprivation for 48 hours. Then supernates were removed. Adherent cells were rinsed three times with PBS and fixed in 3% paraformaldehyde for 20 minutes. After blockade of free aldehyde groups with 50 mmol/L ammonium chloride, MCs were permeabilized by 1% Triton X-100, and nonspecific binding was blocked using 100% FCS. MCs were incubated with the primary antibodies overnight, followed by a CY3-labeled goat anti-rabbit or anti-mouse immunoglobulin G (Dianova) as secondary antibody and embedding in Tris-buffered Mowiol, pH 8.6 (Hoechst).

Antibodies

Antibodies were obtained as follows: Monoclonal antibody to fibrillin-1 was from Chemicon (Hofheim, Germany). Polyclonal (rabbit) as well as monoclonal (mouse) antibodies to human emilin were obtained from rabbits and mice immunized with full-length human recombinant emilin produced by 293-EBNA cells. The specificity of the affinity-purified anti-emilin immunoglobulins was confirmed by immunoblotting against recombinant human emilin (A. Colombatti, unpublished data). The antibody to MAGP-1 was a mouse anti-bovine monoclonal antibody [32], and the MAGP-2 antibody was a rabbit polyclonal raised to a synthetic peptide [21]. Polyclonal antibodies to human elastin were induced in rabbits using human α-elastin as the immunogen (G. Bressan, unpublished data). No cross-reactivity with other fibrillar pro-

teins (fibrillins 1 and 2, emilin, MAGP-1) or other matrix proteins (collagens I, III and VI, fibronectin, laminin) was observed. In addition, staining of rat kidney sections with a commercially available anti-rat antibody to elastin (Chemicon) resulted in an identical staining pattern, confirming specificity of the employed antielastin antibody. Polyclonal antibodies to LTBP-1 were from Pharmingen (Hamburg, Germany), to fibronectin from Life Technologies (Eggenstein, Germany), and to α_8 integrin chain from Dr. Ulrich Müller (Basel, Switzerland) [31, 33]. Monoclonal antibody to Thy 1.1 was from Serotec (Biozol, Eching, Germany). The specificities of the employed antibodies have been described by the respective sources.

Northern blot analysis

RNA of MCs was extracted using the method of Chomczynski and Sacchi [34]. Total RNA was size fractioned on a 1% agarose formaldehyde gel and transferred onto Hybond nylon membranes (Amersham Buchler, Braunschweig, Germany). The blots were baked at 80°C for two hours, prehybridized with 5 × Denhardt's solution, 5 × standard saline citrate (SSC), 50% formamide, 50 mmol/L Na_3PO_4, 0.1% sodium dodecyl sulfate (SDS), 0.25 mg/mL salmon sperm DNA at 40°C for two to four hours. The hybridization probe was amplified by reverse transcription-polymerase chain reaction (RT-PCR) from rat MCs and was labeled with [α-^{32}P] dCTP using a random-primed labeling kit (Rediprime II; Amersham) and column-purified using mini Quick Spin columns (Boehringer Mannheim, Mannheim, Germany). The blots were hybridized in prehybridization solution containing 2×10^6 cpm/mL of probe at 40°C overnight and washed twice for 15 minutes with 2 × SSC containing 0.1% SDS and then 30 minutes with 0.1 × SSC containing 0.1% SDS. As a loading control, a 500 bp GAPDH probe was used [35]. Blots were exposed to Kodak x-ray films with intensifying screens at −80°C for eight hours. The following cDNA probes were employed: rat elastin (clone 124D) comprising 930 bp of the 3′-end of elastin-coding sequence [36]; human emilin, the probe includes the complete coding sequence with the exception of the signal peptide [19]; MAGPs 1 and 2, the clones described by Gibson et al [21]. The cDNAs for human elastin, human fibrillin-1, and murine emilin were cloned by RT-PCR from human placenta total RNA and span the following sequences: human elastin, the entire coding sequence of the mature protein (the signal peptide coding sequence is missing) and 300 bp of 3′-UTR; human fibrillin-1, the 5′-end terminal one fourth of the coding sequence; murine emilin, the 3′-terminal 750 bp of coding sequence and about 100 bp of 3′-UTR (G. Bressan and A. Colombatti, unpublished data).

Effects of TGF-β1 and PDGF in mesangial cell culture

The effects of transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor (PDGF) on cultured MCs were tested with regard to the mRNA expression of the extracellular proteins under study. TGF-β1 and PDGF-BB were obtained from Sigma, and were used at concentrations from 5 to 20 and from 10 to 50 ng/mL, respectively. The growth factors were added to the incubation medium when MC cultures had reached subconfluence and had been switched from 10 to 0.5% FCS for three days to achieve quiescence [35]. Control wells received either vehicle or were supplemented with FCS to reach a final concentration of 10%.

RESULTS

Immunolocalization

Examination of both frozen and paraffin-embedded tissue sections clearly showed that glomeruli of adult mouse, rat, and human kidneys contain multiple extracellular proteins, which are known to be part of or associated with microfibrils and elastic fibers. Depending on the species cross reactivity of the employed antibodies, we found positive glomerular immunostaining by fluorescence and histochemistry for fibrillin-1, emilin, MAGPs 1 and 2, and LTBP-1 (Figs. 1A–C and 2A–G). The glomerular immunoreactivity displayed a mostly mesangial distribution pattern. GBM and the peripheral capillary loop were negative. To confirm mesangial localization of the elastic fiber proteins, we tested for double fluorescence with fibronectin, α_8-integrin and, in the rat, Thy 1.1 antigen, which are proteins known to be expressed in the mesangium [31, 35, 37]. Fibrillin-1, emilin, MAGPs 1 and 2, and LTBP-1 clearly showed glomerular colocalization with fibronectin as well as α_8, confirming their abundance in the mesangium (Fig. 2 E–H). In rat kidney, mesangial colocalization with Thy 1.1 was also found (data not shown). The adventitia and less so the media of renal arteries and arterioles also stained positively for these proteins (Fig. 2G). Immunostaining of interstitial peritubular ECM was observed for fibrillin-1 and emilin (Figs. 1B and 2B, E). Elastin was strongly positive in the vasculature, and, in addition, extended from the glomerular vascular pole to Bowman's capsule and the associated interstitial ECM, but was not appreciably present in glomerular tufts (Fig. 1D). None of the tested antibodies stained the GBM, tubular cells, or basement membranes. These findings are summarized in Table 1.

Electron and immunoelectron microscopy

Following impregnation with tannic acid, a network of electron-dense bundles of microfibrils, 10 to 12 nm in diameter and hollow in cross section, was observed in the

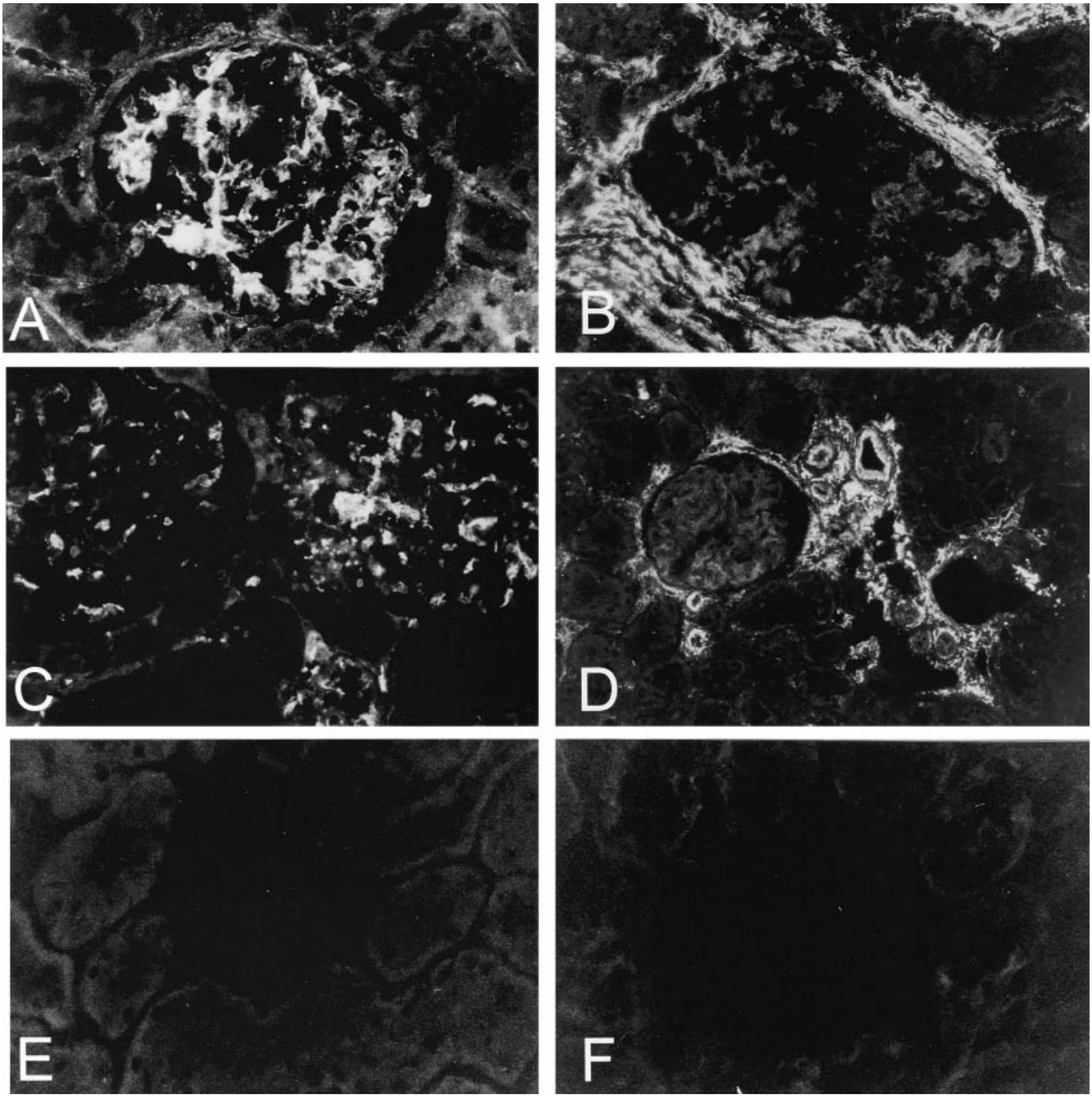

Fig. 1. Immunofluorescence detection of microfibril-associated glycoprotein (MAGP-1; *A*), emilin (*B*), MAGP-2 (*C*), and elastin (*D*) in rat (A, *E*, and *F*) and human frozen kidney tissue (B, C, and D). As negative controls in rat tissue, the monoclonal primary antibodies were replaced by preimmune mouse IgG (E) and the polyclonal antibodies by preimmune rabbit IgG (F).

Fig. 2. Immunohistochemical detection of fibrillin-1 (*A*), emilin (*B*), and latent transforming growth factor-binding protein-1 (LTBP-1; *C*) in rat (A and C) and mouse (B and *D*) paraffin-embedded kidney tissue. As a negative control, the primary antibody was replaced by preimmune mouse IgG (D). Double fluorescence detection of glomerular colocalization of fibrillin-1 (*E*) and fibronectin (*F*) in human frozen kidney tissue or LTBP-1 (*G*) and α8 integrin (*H*) in rat frozen kidney tissue, confirming mesangial localization of fibrillin-1 and LTBP-1.

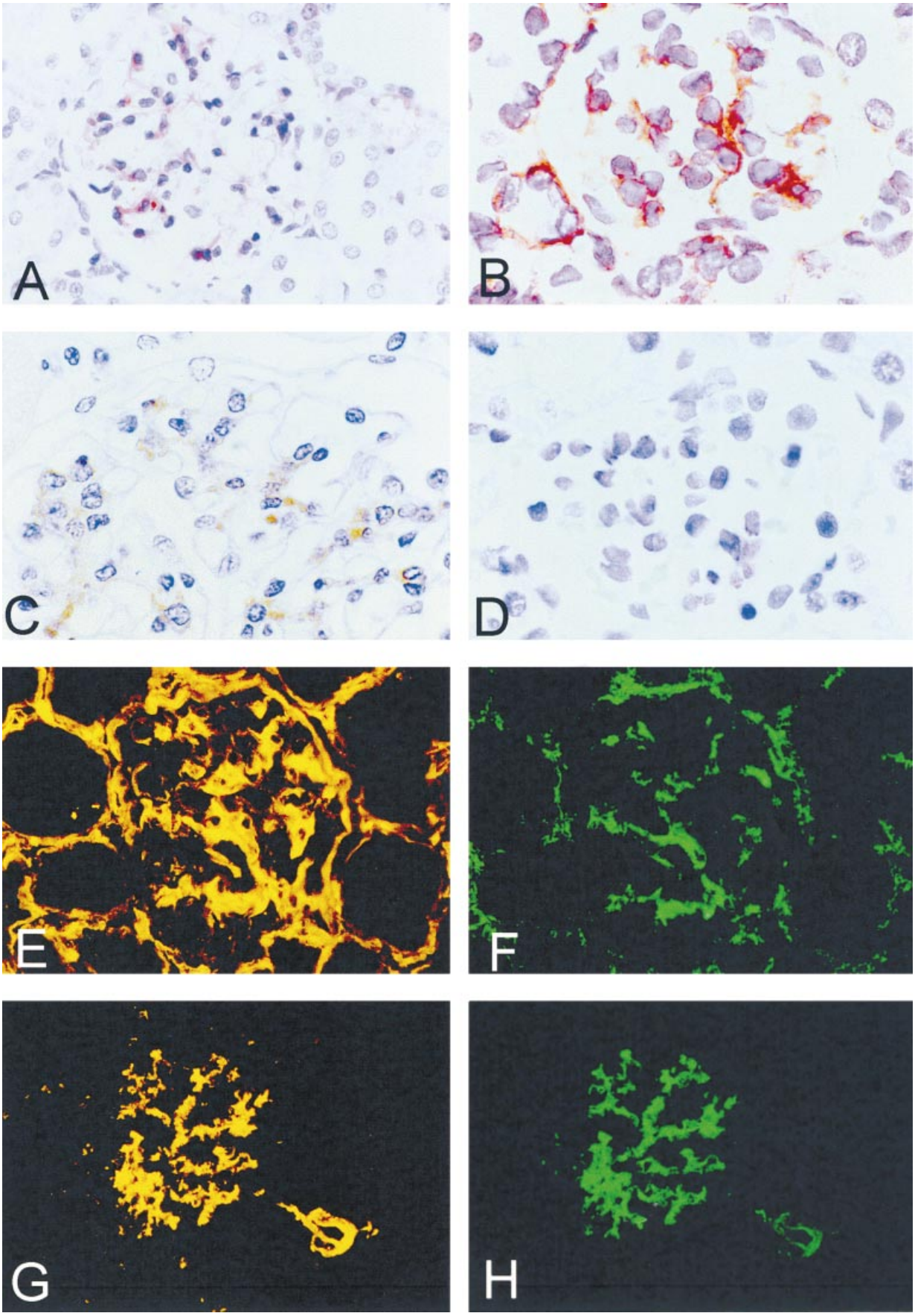
A
B
C
D
E
F
G
H

Table 1. Immunolocalization of elastic fiber proteins in the kidney

	Fibrillin-1	Emilin	MAGP-1	MAGP-2	Elastin	LTBP-1	Fibronectin	α_8-integrin
Glomeruli								
Vascular pole	++	+ to ++	+	++	++	+	+	++
Mesangium	++ to +++	+	++	+ to ++	−	++	+++	+++
Peripheral capillary loop	−	−	−	−	−	−	−	−
GBM	−	−	−	−	−	−	±	−
Bowman's capsule (outer aspect)	−	−	−	−	++	−	−	−
Tubules	−	−	−	−	−	−	−	−
Arterioles								
Subendothelium/media	++	+	±	+	++	++	+	++
Adventitia/perivascular	++	++	−	−	++	++	++	−
Interstitium								
Peritubular	++	+ to ++	+	++	−	+	++	−
Periglomerular	+	+ to ++	+	+	+ to ++	−	++	−

Semiquantitative assessment of immune staining was: − to ±, negative or trace; +, mild; ++, strong; +++, very strong.

Immunolocalization was qualitatively comparable in rat ($N = 4$), mouse ($N = 4$) and human ($N = 3$) kidneys. Scores of staining between species did not differ by more than one grade; scores between kidneys of the same species did not show significant inter-individual variability. Immune staining for elastin in human kidney was negative to trace for Bowman's capsule and strong in periglomerular interstitium. Abbreviations are: MAGP, microfibril-associated glycoprotein; LTBP-1, latent transforming growth factor-binding protein-1; GBM, glomerular basement membrane.

mesangial ECM, which was in accordance with previous findings of Mundel et al (Fig. 3A) [10]. Microfibrils predominantly localized subendothelially at the mesangial angles of glomerular capillaries. They appeared to insert within membrane invaginations of MCs and to connect with the inner aspect of the GBM (Fig. 3B).

At the ultrastructural level, epitopes for fibrillin-1, emilin, MAGP-2, and LTBP-1 could be demonstrated by immunogold labeling in the mesangial ECM. Antibodies to fibrillin-1 showed the strongest immunoreactivity (Fig. 3 B–F), while weaker reactivities were found for emilin, MAGP-2, and LTBP-1 (Fig. 4). The latter became evident only after pretreatment of sections with proteases and microwave heating or by use of pre-embedding labeling. The antibody to MAGP-1 did not show immunogold labeling in kidney specimens. The gold marker for fibrillin-1 was clearly associated with the delicate microfibrillar bundles, which appeared embedded in the mesangial ECM, particularly in the subendothelial position (Fig. 3B). In some areas, microfibrils appeared attached with membranes of MC processes or to extend to endothelial cells or to insert into the inner aspects of the GBM (Fig. 3C–E). Tannic acid impregnation improved visualization of the fibrillin-1–positive microfibrils (Fig. 3F). The gold markers for MAGP-2 and LTBP-1 also immunolocalized to microfibrillar bundles attached to MCs (Fig. 4A, B), whereas emilin epitopes appeared scattered throughout the mesangial ECM (Fig. 4C). The GBM proper, cytoplasm, nuclei of glomerular cells, and tubuli were completely negative with all antibodies employed.

In the peritubular connective tissue, isolated bundles of microfibrils, occasionally in association with interstitial fibroblasts, could also be immunolabeled with the employed antibodies (Fig. 4D). In addition, emilin localized to the periphery of elastic fibers (Fig. 4E). In the renal vasculature, gold-labeled bundles localized emilin predominantly in the subendothelium and to the adventitia as well as to elastic fibers found in the perivascular connective tissue. In the human kidney, the gold marker for elastin was restricted to prominent elastic fiber cords surrounding Bowman's capsule (Fig. 4F) and to vascular structures.

Studies of mesangial cell cultures

To analyze not only the presence and expression, but also production and regulation of elastic fiber proteins in defined glomerular cells, we performed studies with MCs in primary culture. Regulatory effects related to MC proliferation and caused by growth-modulating factors on the expression at the mRNA and protein levels were also examined. MCs of rats were used in the majority of experiments. For specific questions, human and murine cells were also analyzed.

Immunocytochemistry

Using immunofluorescence, MCs in two-dimensional culture revealed positive staining for all tested proteins. Immune staining for emilin, MAGP-2, and elastin is shown in Figure 5A–E. As far as the cross-reactive antibodies were available, we found no clear-cut species differences in immunostaining of rat versus mouse versus human MCs. Positive cellular staining was found when MCs were sparsely seeded (day 1) or subconfluent (days 3 through 6). With time and increasing cell density (confluence by days 6 through 8), strong extracellular immunostaining was observed, often appearing in irregular fibers and ultimately forming a dense fibrillar network extending throughout the culture chamber (Fig. 5C, E).

mRNA expression in cultured mesangial cells

Northern blot analysis demonstrated that rat MCs express mRNAs of fibrillin-1, emilin, and elastin (Fig. 6).

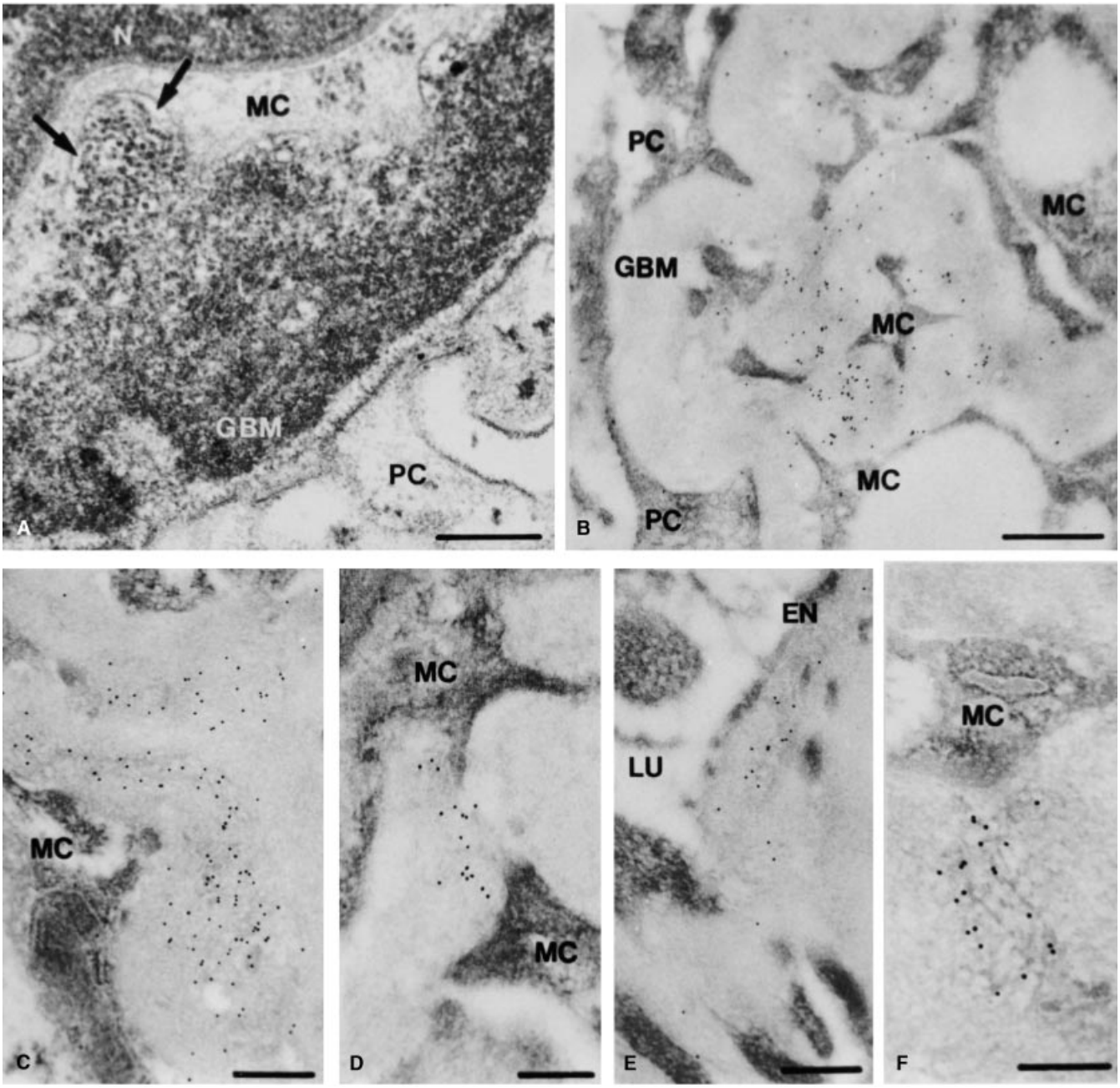

Fig. 3. Transmission electron microscopy of microfibrillar bundles in the glomerular mesangial extracellular membrane (ECM) of human kidney tissue visualized by tannic acid impregnation (*A*) and fibrillin-1 immunolabeling (*B–F*). (A) Microfibrillar bundle in cross section (arrows) between a mesangial cell (MC) and the glomerular basement membrane (GBM) after tannic acid impregnation. Abbreviations are: N, nucleus; PC, podocyte foot processes (bar = 0.3 μm). (B) Positive staining for fibrillin-1 in the angle, where the endothelial (EN)–mesangial (MC) interface meets the GBM (bar = 0.5 μm). (C) Fibrillin-1–positive microfibrillar bundles attached to a MC in detail (bar = 0.3 μm). (D) Fibrillin-1–positive microfibrillar bundle between two MC processes (bar = 0.2 μm). (E) Subendothelial microfibrillar bundle labelled for fibrillin-1 (LU, capillary lumen; bar = 0.3 μm). (F) After tannic acid impregnation, the microfibrillar nature of the fibrillin-1–positive structures attached to MCs is more easily recognized (bar = 0.2 μm).

Their expression was strong when MCs had been maintained in 0.5% FCS-containing medium for three days; that is, when cells are quiescent and more differentiated (Fig. 6). Supplementation of subconfluent quiescent MC cultures with 10% FCS caused a rather prompt decline of gene expression lasting from 2 to 24 hours (Fig. 6). This suppressive effect was most pronounced for emilin and elastin mRNA and was weak for fibrillin-1. However, mRNA expression of the tested proteins recovered after two or three days in 10% FCS when MC cultures

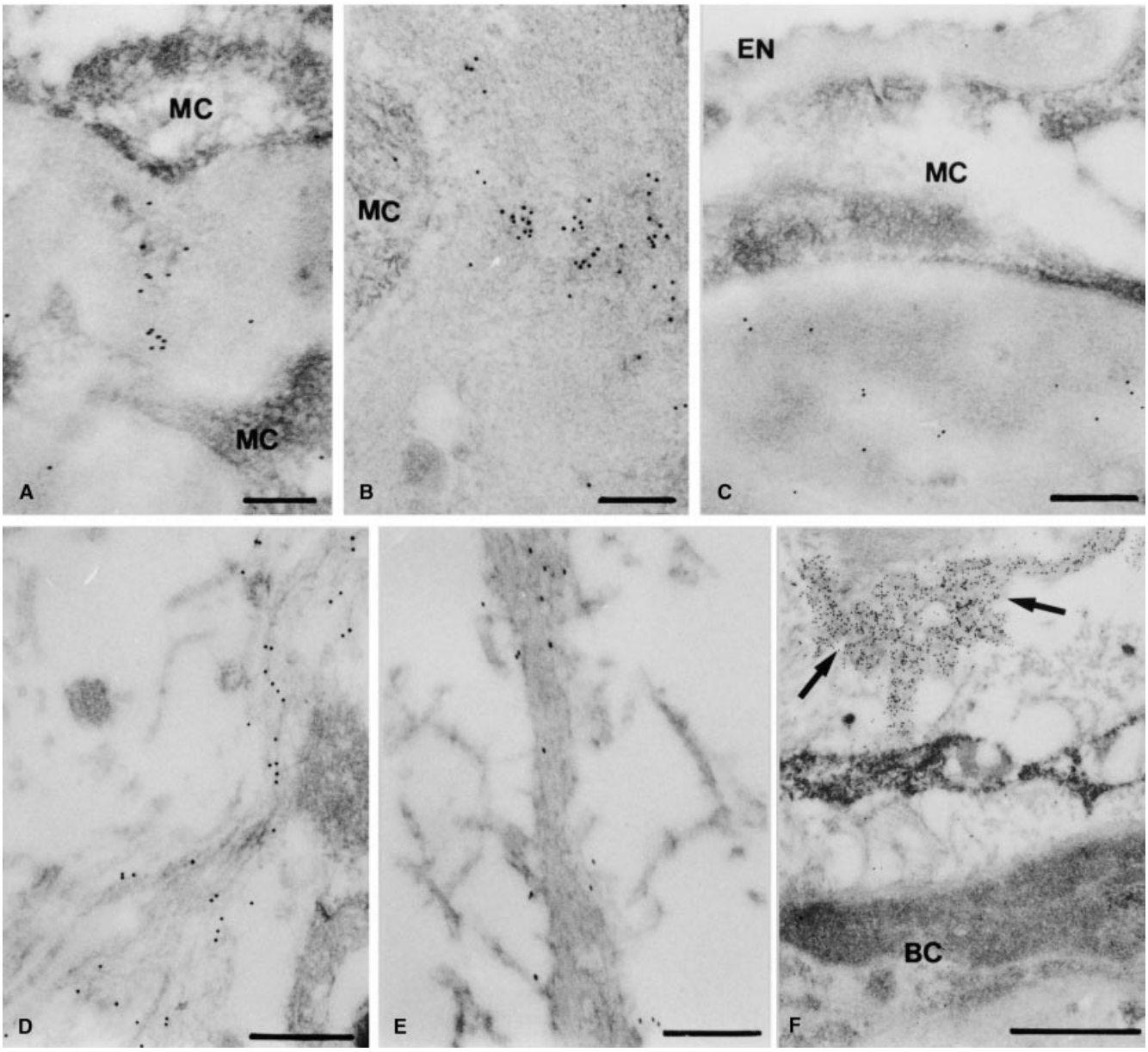

Fig. 4. Transmission electron micrographs showing immunogold labeling for MAGP-2 (*A*), LTBP-1 (*B*), emilin (*C–E*), and elastin (*F*) in the mesangial ECM and peritubular and periglomerular connective tissue of human kidney specimens. (A) MAGP-2 immunoreactivity of a microfibrillar bundle connecting two MC processes (bar = 0.2 μm). (B) The gold marker for LTBP-1 is associated with a microfibrillar bundle close to a MC (trypsin pretreatment; bar = 0.2 μm). (C) Immunolabeling for emilin is scattered in the mesangial ECM (EN endothelium; bar = 0.3 μm). (D and E) Immunolocalization of emilin to microfibrillar bundles (D) and to the periphery of elastic fibers (E) in the interstitial connective tissue (bars = 0.3 μm). (F) An elastin-positive elastic fiber (arrows) in the connective tissue close to Bowman's capsule (BC; bar = 1 μm).

reached confluence because of the strong mitogenic stimulus and proliferative burst of MCs. Such mRNA recovery was not observed when initially sparsely seeded MC cultures did not reach confluence despite the presence of 10% FCS (data not shown). The addition of TGF-β1 (5 ng/mL) to the supernate of quiescent MCs further augmented the mRNA expression of all tested proteins in a time-dependent fashion, which was particularly obvious for elastin (Fig. 6). The up-regulating effect of TGF-β1 was also dose dependent (data not shown), being strongest at a concentration of 20 ng/mL.

Platelet-derived growth factor-BB caused early mRNA induction at two to eight hours, but this was followed by decrease of gene expression of the tested proteins (Fig. 7). In rat MCs, the down-regulating effect of PDGF was most pronounced for mRNA expression of elastin and was weaker or more transitory for emilin and fibrillin-1 (Fig. 7).

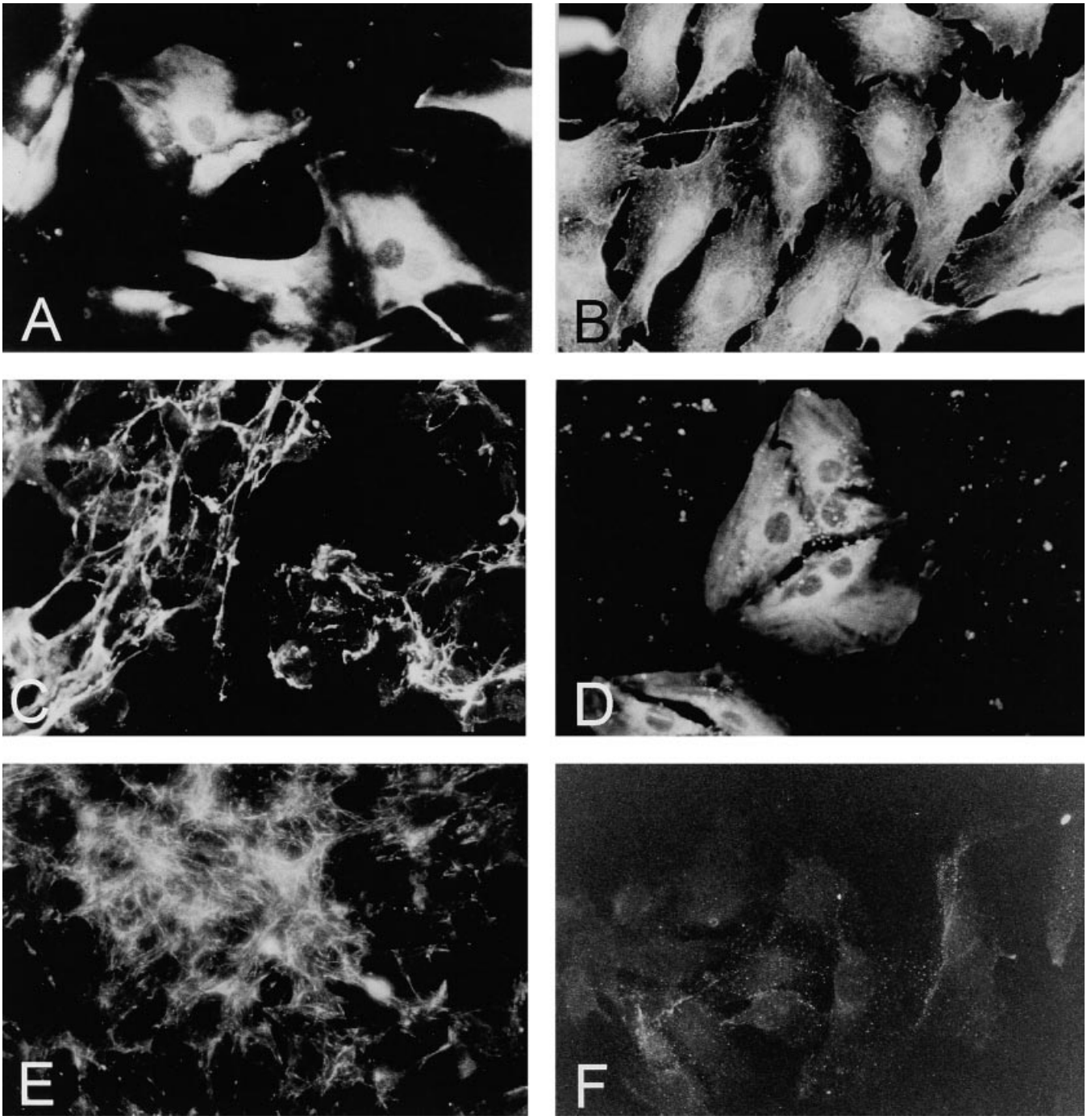

Fig. 5. Immunocytochemical detection of emilin (*A*, *C*, and *E*), MAGP-2 (*B*), and elastin (*D*) in cultured rat MCs. Staining for emilin in MC cultures with increasing cell density is seen in A, C, and E, showing extracellular accumulation of emilin. Incubation with preimmune mouse IgG instead of the primary antibody served as a negative control (*F*). Photomicrographs show representative immunocytochemical results obtained in three separate sets of MC culture experiments.

In addition to the experiments on gene expression in rat MCs, analogous studies were also performed in human and murine MCs. With the available cDNA probes, we found qualitatively similar mRNA expression for fibrillin-1, emilin, and elastin in MCs from human and mouse kidneys. Moreover, we observed analogous up-regulatory effects of TGF-β1 and inhibitory effects of PDGF in mouse (Fig. 8) and human (data not shown) MCs. The mRNA expression of MAGPs 1 and 2 could only be detected in human MCs. The employed human probes did not cross react with rat or mouse RNA (data not shown).

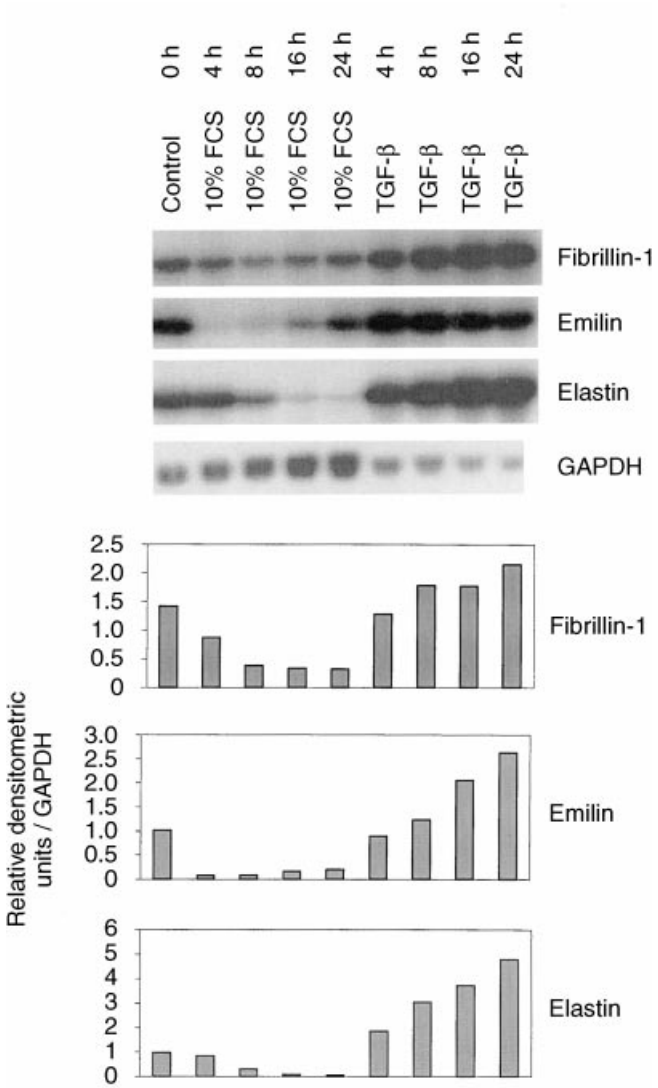

Fig. 6. Time-dependent regulation of fibrillin-1, emilin, and elastin mRNA expression in cultured rat MCs by 10% FCS and TGF-β1 (5 ng/mL), as detected by Northern blot analysis. Control, MCs in 0.5% FCS. Densitometric evaluation of blots is depicted in the lower panels. Representative results of three independent experiments are shown.

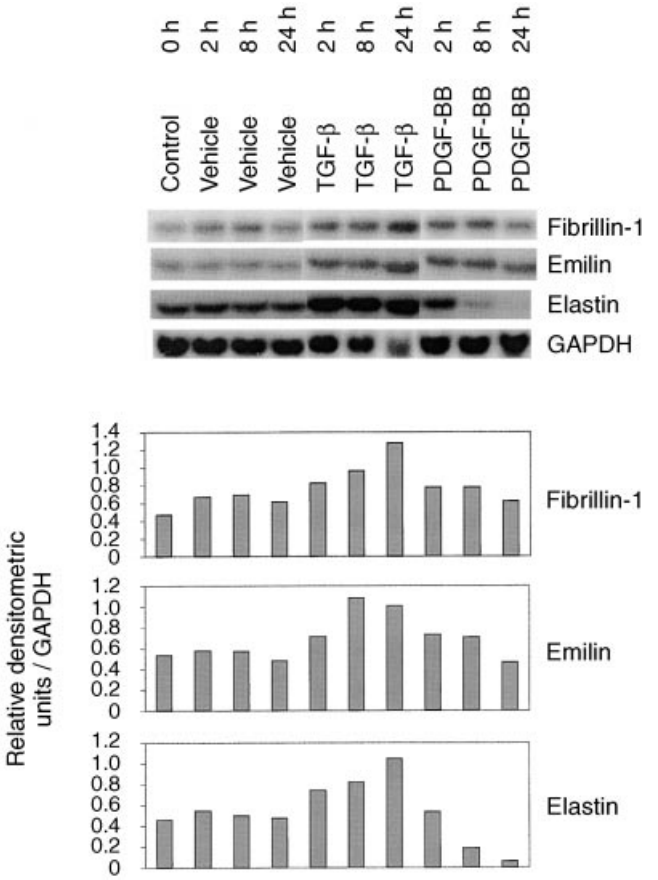

Fig. 7. Regulation of fibrillin-1, emilin, and elastin mRNA expression in rat mesangial cells (MCs) by transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor-BB (PDGF-BB), as detected by Northern blot analysis. Control, MCs in 0.5% FCS. Time-dependent effects of vehicle (4 mmol/L HCl/0.1% BSA) and 20 ng/mL PDGF-BB. Densitometric evaluation of blots is depicted in the lower panels. Data shown are representative for four independent experiments.

DISCUSSION

The results of the present study demonstrate that the mesangial ECM of normal human, rat, and mouse glomeruli contains multiple proteins that are constituents of or associated with the microfibrillar component of elastic fibers. They include the glycoproteins fibrillin-1, emilin, MAGPs 1 and 2, as well as LTBP-1. Immunoelectron microscopy revealed that fibrillin-1–containing microfibrils are localized in the mesangial ECM, particularly in the subendothelium at the endothelial–mesangial interface and where it underlies the paramesangial GBM. Emilin, MAGP-2, and LTBP-1 showed similar albeit weaker immunoreactivities. In primary cultures of MCs from rat, mouse, and human glomeruli, these proteins are abundantly expressed, as shown by immunocytochemistry and Northern blot analysis. Their gene expression is stronger in quiescent than in actively proliferating MCs, and it is up-regulated by TGF-β1.

Microfibril-associated proteins in elastic tissues

Our findings confirm and extend previous observations obtained by immunofluorescence microscopy, which showed that microfibrillar proteins, such as fibrillin-1 and MAGPs 1 and 2, are present in glomeruli as well as in the vasculature of adult chick, bovine, human, and rat kidney [20, 21, 23, 38]. Gibson et al identified several microfibrillar components by biochemical and ultrastructural analysis, and described their distribution in skin and tendons as well as in the general vasculature, including the kidney [13, 21, 24]. The results of these and other investigations suggested that fibrillin-1 is the main component of extensible microfibrils, which contribute

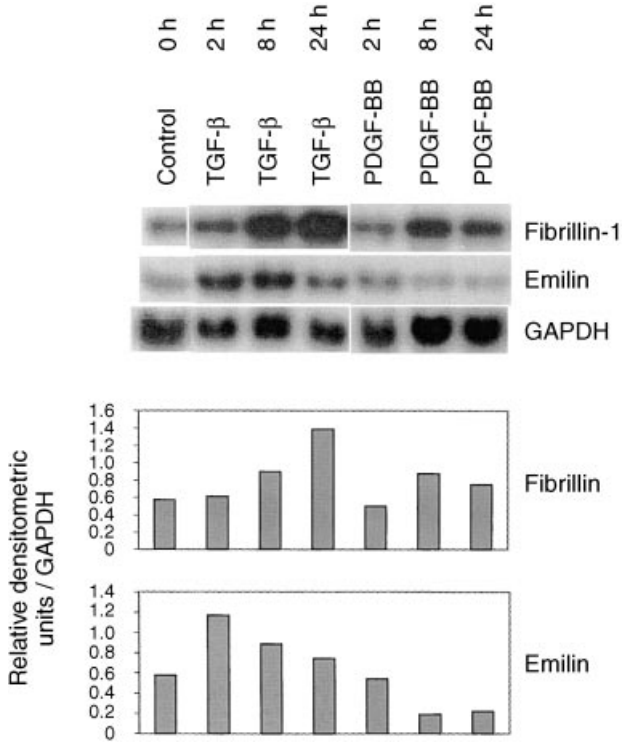

Fig. 8. Regulation of fibrillin-1 and emilin mRNA expression in mouse MCs by TGF-β1 and PDGF-BB, as detected by Northern blot analysis. Control, MCs in 0.5% FCS. Time kinetics of mRNA expression of fibrillin-1 and emilin after stimulation with 5 ng/mL TGF-β1 or 10 ng/mL PDGF-BB. Densitometric evaluation of blots is depicted in the lower panels. Representative results of three MC culture experiments are shown.

to the elastic strength of tissues subjected to mechanical stress [13, 14, 16, 21, 39]. The associated proteins, for example, MAGPs 1 and 2, also provide anchorage by connecting microfibrils and elastic fibers among themselves and with collagens, for example, of basement membranes, as well as by linking this ECM network to adjacent cells [14, 16, 21, 22, 24, 39]. The glycoprotein emilin is located predominantly at the interface between microfibrils and elastin, but is also found in the absence of elastin [20]. At present, the function of emilin is not precisely known. Recent studies indicated that emilin serves adhesive and migratory functions for various cell types, including MCs (Colombatti et al, unpublished data) [19]. Whereas elastin is the most important elastic protein, its presence does not appear to be essential for the elastic strength of networks of extensible microfibrils containing fibrillin-1 and MAGP-2, as observed in the subendothelium and skin [14, 16, 21, 39]. Thus, the available results are in keeping with the interpretation that microfibril-related proteins can serve elastic as well as nonelastic functions of the ECM.

Microfibrils in the mesangium

Several investigators have provided ultrastructural evidence showing microfibrillar structures (10 to 15 nm in width) in the mesangial ECM of mammalian glomeruli [1, 2, 11, 12]. Extensive studies by Kriz et al demonstrated the particular localization of such microfibrils in the ECM at the junction of the endothelial–mesangial interface and GBM as well as underneath the paramesangial GBM [1, 2, 7, 10]. These ultrastructural findings, together with functional studies by others [3, 9], point to the relevance of the mesangial ECM to provide elastic constraint to the glomerular capillary tuft, thus counterbalancing (in concert with the tubulointerstitial pressure) the high intraglomerular hydrostatic pressure and preventing inappropriate distention of the capillary wall.

The immunohistochemical results of the present study are supported by immunoelectron microscopy findings, which clearly demonstrate that the glomerular localization of fibrillin-1, MAGP-2, LTBP-1, and emilin is restricted to the mesangial ECM, sparing the GBM (Figs. 3 and 4). We found mesangial colocalization of these proteins with fibronectin and α_8-integrin (Fig. 2E–H). These observations in conjunction with reports by others [2, 3, 19, 31, 38] indicate that the mesangial ECM is composed of a complex array of extracellular proteins serving different functions, including mechanical stabilization and elastic strength of the glomerular capillary tuft. Of note, we did not find elastin localized in the glomerular capillary tuft but only in the renal vasculature, including the vascular pole at the glomerular hilus, extending from there to Bowman's capsule and the associated interstitial ECM (Fig. 1D and Table 1). These findings are consistent with the concept that a network of elastin-free microfibrillar bundles in the mesangial ECM serves stress-bearing and elastic functions for the maintenance of the glomerular capillary tuft in addition to providing anchorage of the mesangium to the GBM. By analogy, elastin-free microfibrils found in the superficial layer of the dermis and in the vascular subendothelium are also thought to provide elastic strength [14, 16, 39]. However, the precise macromolecular architecture and elastic properties of extensible microfibrils in the absence of elastin are presently unknown. The continuous circumferential localization of elastin associated with the interstitial aspect of Bowman's capsule is remarkable. It could play a role in the mechanical maintenance of Bowman's space, the initial portion of the nephron, for example, under conditions causing intratubular obstruction or reflux with enhanced intratubular hydrostatic pressure. Similarly, the peritubular abundance of elastic fiber proteins, such as fibrillin-1, emilin, and MAGPs 1 and 2 could serve analogous functions, that is, opposing distending forces when intratubular pressure increases. However, detailed analysis of the structure–function relationships is needed to answer questions concerning the potential role of specific elastic fiber proteins in nephron anchorage and resilience.

Microfibril-associated proteins in the mesangial ECM

The abundance of LTBP-1 in the mesangial ECM of normal mammals was previously observed by others [25, 27, 28]. Interestingly, LTBP-1 was recently described to be part of microfibrils, for example, in the skin and other tissues [16, 26, 40]. It is presently uncertain whether this protein also serves mechanical functions of the mesangial ECM besides being a potential extracellular binding protein for latent TGF-β, thus affecting the regulation of active TGF-β [25, 28]. Further investigations are required to discern the diverse functional properties and interactions of other elastic fiber proteins present in the mesangial ECM. Fibrillins 1 and 2 as well as MAGP-2 have been found to ligate to $\alpha_v\beta_3$ integrin receptors of various mesenchymal cell types [16–18, 41–43]. Also, fibrillin-1 can bind to components of the basement membrane, such as fibulin-2 [23]. It is presently unclear whether such linkage potential of elastic fiber proteins also holds for the glomerular capillary tuft. In normal kidney tissue, MCs express multiple integrins of the β_1 family, notably α_1, α_2, α_3 and α_8, but not $\alpha_v\beta_3$ [31, 37]. However, mesangial α_v expression is greatly up-regulated in glomerular disease [19, 44]. We expect that elucidation of the nature of elastic fiber protein ligation to MCs as well as to components of the GBM will be useful to understand better how MCs transmit supportive and contractile forces to the glomerular capillaries.

Production of elastic fiber proteins by cultured mesangial cells

By immunocytochemistry of cultured MCs, we obtained direct evidence that MCs have the potential to produce fibrillin-1, emilin, MAGPs 1 and 2, LTBP-1, as well as elastin. We performed additional analyses by Northern blotting using cDNA probes that were available to us. The results of these short-term experiments confirm that MCs express mRNAs of fibrillin-1, emilin, MAGPs 1 and 2, and elastin (Fig. 5). Their expression is regulated in that it is strong in MCs maintained in 0.5% FCS for three days when proliferative activity of MCs is low and cells have reached a more quiescent state [35]. Gene expression is further up-regulated by TGF-β1 during the course of three consecutive days, whereas it is transiently reduced or abolished in the presence of mitogenic stimuli, such as 10% FCS and PDGF. The stimulating effects of TGF-β1 are of interest because its expression can be induced by exposure of cultured MCs to cyclic stretch [45]. Thus, it appears possible that in vivo, TGF-β1 is involved in the up-regulation of elastic fiber proteins, together with other ECM components, for example, in response to enhanced mechanical stress to glomerular capillaries. At present, we do not know the signaling pathways by which the expression of the studied proteins is controlled in MCs, for example, by growth factors. Recent reports by Ahmed et al and Kucich et al have shown that in fetal lung fibroblasts, the up-regulating effects of TGF-β1 on various elastic fiber components, can occur at the level of transcription or of message stabilization [46, 47]. Which of such diverse mechanisms are utilized in MCs and what relevance they may have in vivo require further analysis.

It is of interest that elastin mRNA and protein are also markedly expressed in cultured MCs, whereas we failed to demonstrate this protein by immunolocalization in the intraglomerular mesangium in renal tissue sections (Figs. 1D and 5). A possible explanation for this discrepancy could be that even in medium containing merely 0.5% FCS, MCs kept in two-dimensional culture for up to six days are not truly differentiated as they are under normal in vivo conditions. It remains to be seen whether elastin protein stays expressed by quiescent and more differentiated MCs in three-dimensional culture and whether it becomes apparent in the mesangium of diseased glomeruli when MCs are activated.

Fibrillin-1 deficiency and function

Multiple mutations of the fibrillin-1 gene have been described in patients with the pleiomorphic Marfan syndrome [48, 49]. First studies were recently reported on fibrillin-1 gene-mutated mice with partial inhibition of gene expression resulting in shortened fibrillin-1 protein or with underexpression of fibrillin-1 [16, 40, 49]. Depending on the gene defect, these mice die within the first few months after birth because of cardiovascular complications caused by mechanical collapse and dissecting aneurysms of the aortic wall. Whereas fibrillin-1–deficient mice showed other hallmarks of Marfan syndrome, no obviously abnormal renal phenotype was observed in their short life span [40, 49]. Kanwar et al discovered that the administration of fibrillin-1 antisense oligonucleotides to rat embryonic metanephroi in culture greatly disturbed renal development [38]. The authors suggested that the ECM ligand fibrillin-1 could play a role in mammalian renal organogenesis, for example, in the vascularization phase of the metanephric kidney [38, 50]. Besides noting glomerular immunostaining for fibrillin-1 in embryonic and postnatal kidneys, no mention of glomerular development was made [38]. While several anecdotal reports of different types of glomerular disease in Marfan patients have appeared [51, 52], no systematic study has yet been published allowing clues as to the relationships between particular mutations of the fibrillin-1 gene, defects of fibrillin-1 protein and pathology of renal vasculature and glomeruli in such patients, for example, in autopsy studies. In the future, it will be interesting to examine Marfan patients as well as experimental animals with established fibrillin-1 abnormalities as to the prevalence and nature of renal pathology.

Finally, further investigations are required to elucidate whether MC exposure to other stimuli, such as mechanical stretch or vasoactive substances (for example, angiotensin II and endothelin-1) affect the mesangial expression of elastic fiber proteins. Based on the present results, it appears reasonable to test the MC expression, abundance, regulation, and potential function of elastic fiber proteins found in the mesangial ECM, for example, in MC culture and in animal experiments, in which the determinants of the glomerular microcirculation or the size and volume of glomeruli are altered. Such studies should help to clarify what role elastic fiber proteins of the mesangial ECM play in the normal homeostasis of structure and function of the glomerular capillary tuft and in diseases associated with glomerular hypertension and/or hypertrophy.

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REFERENCES

1. Kriz W, Elger M, Mundel P, Lemley KV: Structure-stabilizing forces in the glomerular tuft. (editorial) *J Am Soc Nephrol* 5:1731–1739, 1995
2. Kriz W, Elger M, Lemley K, Sakai T: Structure of the glomerular mesangium: A biomechanical interpretation. *Kidney Int* 38(Suppl 30):S2–S9, 1990
3. Cortes P, Zhao X, Riser BL, Narins RG: Regulation of glomerular, in normal and partially nephrectomized rats. *Am J Physiol* 270:F356–F370, 1996
4. Dworkin LD, Brenner BM: Biophysical basis of glomerular filtration, in *The Kidney: Physiology and Pathophysiology*, edited by Seldin DW, Giebisch G, New York, Raven, 1992, p 979
5. Meyer TM, Baboolal K, Brenner BM: Nephron adaptation to renal injury, in *The Kidney*, edited by Brenner BM, Philadelphia, Saunders, 1996, pp 2011–2048
6. Drumond MC, Deen WM: Analysis of pulsatile pressures and flows in glomerular filtration. *Am J Physiol* 261:F409–F419, 1991
7. Sakai T, Kriz W: The structural relationship between mesangial cells and basement membrane of the renal glomerulus. *Anat Embryol (Berl)* 176:373–386, 1987
8. Kriz W, Gretz N, Lemley KV: Progression of glomerular diseases: Is the podocyte the culprit? *Kidney Int* 54:687–697, 1998
9. Riser BL, Cortes P: The nature of the diabetic glomerulus: Pressure-induced and metabolic aberrations, in *The Kidney and Hypertension in Diabetes Mellitus*, edited by Mogensen CE, Boston, Kluwer Academic Publishers, 1998
10. Mundel P, Elger M, Sakai T, Kriz W: Microfibrils are a major component of the mesangial matrix in the glomerulus of the rat kidney. *Cell Tissue Res* 254:183–187, 1988
11. Farquhar MG, Palade GE: Functional evidence for the existence of a third cell type in the renal glomerulus: Phagocytosis of filtration residues by a distinctive "third" cell. *J Cell Biol* 13:55–87, 1962
12. Reale E, Luciano L, Kuhn KW, Stolte H: Morphological and functional aspects of the glomerular basement membrane. *Basic Appl Histochem* 23(Suppl):5–11, 1979
13. Cleary EG, Gibson MA: Elastic tissue, elastin and elastin associated microfibrills, in *Molecular Components and Interactions*, edited by Comper WD, Amsterdam, Harwood Academic, 1996, pp 95–140
14. Fauvel-Lafeve F: Microfibrils from the arterial subendothelium. *Int Rev Cytol* 188:1–40, 1999
15. Rosenbloom J: Elastin, in *Connective Tissue and its Heritable Disorders*, New York, Wiley-Liss, 1993, pp 167–188
16. Ramirez F, Pereira L: The fibrillins. *Int J Biochem Cell Biol* 31: 255–259, 1999
17. Reinhardt DP, Keene DR, Corson GM, Poschl E, Bachinger HP, Gambee JE, Sakai LY: Fibrillin-1: Organization in microfibrils and structural properties. *J Mol Biol* 258:104–116, 1996
18. Yang Q, Ota K, Tian Y, Kumar A, Wada J, Kashihara N, Wallner E, Kanwar YS: Cloning of rat fibrillin-2 cDNA and its role in branching morphogenesis of embryonic lung. *Dev Biol* 212: 229–242, 1999
19. Doliana R, Mongiat M, Bucciotti F, Giacomello E, Deutzmann R, Volpin D, Bressan GM, Colombatti A: EMILIN, a component of the elastic fiber and a new member of the C1q/tumor necrosis factor superfamily of proteins. *J Biol Chem* 274:16773–16781, 1999
20. Bressan GM, Daga-Gordini D, Colombatti A, Castellani I, Marigo V, Volpin D: Emilin, a component of elastic fibers preferentially located at the elastin-microfibrils interface. *J Cell Biol* 121:201–212, 1993
21. Gibson MA, Finnis ML, Kumaratilake JS, Cleary EG: Microfibril-associated glycoprotein-2 (MAGP-2) is specifically associated with fibrillin-containing microfibrils but exhibits more restricted patterns of tissue localization and developmental expression than its structural relative MAGP-1. *J Histochem Cytochem* 46:871–886, 1998
22. Kumaratilake JS, Gibson MA, Fanning JC, Cleary EG: The tissue distribution of microfibrils reacting with a monospecific antibody to MAGP, the major glycoprotein antigen of elastin-associated microfibrils. *Eur J Cell Biol* 50:117–127, 1989
23. Reinhardt DP, Sasaki T, Dzamba BJ, Keene DR, Chu ML, Gohring W, Timpl R, Sakai LY: Fibrillin-1 and fibulin-2 interact and are colocalized in some tissues. *J Biol Chem* 271:19489–19496, 1996
24. Gibson MA, Kumaratilake JS, Cleary EG: Immunohistochemical and ultrastructural localization of MP78/70 (betaig-h3) in extracellular matrix of developing and mature bovine tissues. *J Histochem Cytochem* 45:1683–1696, 1997
25. Ando T, Okuda S, Tamaki K, Yoshitomi K, Fujishima M: Localization of transforming growth factor-beta and latent transforming growth factor-beta binding protein in rat kidney. *Kidney Int* 47:733–739, 1995
26. Raghunath M, Unsold C, Kubitscheck U, Bruckner-Tuderman L, Peters R, Meuli M: The cutaneous microfibrillar apparatus contains latent transforming growth factor-beta binding protein-1 (LTBP-1) and is a repository for latent TGF-beta 1. *J Invest Dermatol* 111:559–564, 1998
27. Taipale J, Saharinen J, Hedman K, Keski-Oja J: Latent transforming growth factor-beta 1 and its binding protein are components of extracellular matrix microfibrils. *J Histochem Cytochem* 44:875–889, 1996
28. Hori Y, Katoh T, Hirakata M, Joki N, Kaname S, Fukagawa M, Okuda T, Ohashi H, Fujita T, Miyazono K, Kurokawa K: Anti-latent TGF-beta binding protein-1 antibody or synthetic oligopeptides inhibit extracellular matrix expression induced by stretch in cultured rat mesangial cells. *Kidney Int* 53:1616–1625, 1998
29. Schlotzer-Schrehardt U, von der Mark K, Sakai LY, Naumann GO: Increased extracellular deposition of fibrillin-containing fibrils in pseudoexfoliation syndrome. *Invest Ophthalmol Vis Sci* 38:970–984, 1997
30. Rupprecht HD, Dann P, Sukhatme VP, Sterzel RB, Coleman DL: Effect of vasoactive agents on induction of Egr-1 in rat mesangial cells: Correlation with mitogenicity. *Am J Physiol* 263:F623–F636, 1992
31. Hartner A, Schocklmann H, Prols F, Muller U, Sterzel RB:

Alpha8 integrin in glomerular mesangial cells and in experimental glomerulonephritis. *Kidney Int* 56:1468–1480, 1999
32. Henderson M, Polewski R, Fanning JC, Gibson MA: Microfibril-associated glycoprotein-1 (MAGP-1) is specifically located on the beads of the beaded-filament structure for fibrillin-containing microfibrils as visualized by the rotary shadowing technique. *J Histochem Cytochem* 44:1389–1397, 1996
33. Muller U, Wang D, Denda S, Meneses JJ, Pedersen RA, Reichardt LF: Integrin alpha8beta1 is critically important for epithelial-mesenchymal interactions during kidney morphogenesis. *Cell* 88:603–613, 1997
34. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
35. Schoecklmann HO, Rupprecht HD, Zauner I, Sterzel RB: TGF-beta1-induced cell cycle arrest in renal mesangial cells involves inhibition of cyclin E-cdk 2 activation and retinoblastoma protein phosphorylation. *Kidney Int* 51:1228–1236, 1997
36. Deak SB, Pierce RA, Belsky SA, Riley DJ, Boyd CD: Rat tropoelastin is synthesized from a 3.5-kilobase mRNA. *J Biol Chem* 263:13504–13507, 1988
37. Prols F, Hartner A, Schocklmann HO, Sterzel RB: Mesangial cells and their adhesive properties. *Exp Nephrol* 7:137–146, 1999
38. Kanwar YS, Ota K, Yang Q, Kumar A, Wada J, Kashihara N, Peterson DR: Isolation of rat fibrillin-1 cDNA and its relevance in metanephric development. *Am J Physiol* 275:F710–F723, 1998
39. Kielty CM, Shuttleworth CA: Microfibrillar elements of the dermal matrix. *Microsc Res Tech* 38:413–427, 1997
40. Pereira L, Lee SY, Gayraud B, Andrikopoulos K, Shapiro SD, Bunton T, Biery NJ, Dietz HC, Sakai LY, Ramirez F: Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. *Proc Natl Acad Sci USA* 96:3819–3823, 1999
41. Pfaff M, Reinhardt DP, Sakai LY, Timpl R: Cell adhesion and integrin binding to recombinant human fibrillin-1. *FEBS Lett* 384: 247–250, 1996
42. Sakamoto H, Broekelmann T, Cheresh DA, Ramirez F, Rosenbloom J, Mecham RP: Cell-type specific recognition of RGD- and non-RGD-containing cell binding domains in fibrillin-1. *J Biol Chem* 271:4916–4922, 1996
43. Gibson MA, Leavesley DI, Ashman LK: Microfibril-associated glycoprotein-2 specifically interacts with a range of bovine and human cell types via alphaVbeta3 integrin. *J Biol Chem* 274:13060–13065, 1999
44. Shikata K, Makino H, Morioka S, Kashitani T, Hirata K, Ota Z, Wada J, Kanwar YS: Distribution of extracellular matrix receptors in various forms of glomerulonephritis. *Am J Kidney Dis* 25: 680–688, 1995
45. Riser BL, Cortes P, Heilig C, Grondin J, Ladson-Wofford S, Patterson D, Narins RG: Cyclic stretching force selectively upregulates transforming growth factor-beta isoforms in cultured rat mesangial cells. *Am J Pathol* 148:1915–1923, 1996
46. Ahmed W, Kucich U, Abrams W, Bashir M, Rosenbloom J, Segade F, Mecham R: Signaling pathway by which TGF-beta1 increases expression of latent TGF- beta binding protein-2 at the transcriptional level. *Connect Tissue Res* 37:263–276, 1998
47. Kucich U, Rosenbloom JC, Shen G, Abrams WR, Blaskovich MA, Hamilton AD, Ohkanda J, Sebti SM, Rosenbloom J: Requirement for geranylgeranyl transferase I and acyl transferase in the TGF-beta-stimulated pathway leading to elastin mRNA stabilization. *Biochem Biophys Res Commun* 252:111–116, 1998
48. Eldadah ZA, Brenn T, Furthmayr H, Dietz HC: Expression of a mutant human fibrillin allele upon a normal human or murine genetic background recapitulates a Marfan cellular phenotype. *J Clin Invest* 95:874–880, 1995
49. Pereira L, Andrikopoulos K, Tian J, Lee SY, Keene DR, Ono R, Reinhardt DP, Sakai LY, Biery NJ, Bunton T, Dietz HC, Ramirez F: Targeting of the gene encoding fibrillin-1 recapitulates the vascular aspect of Marfan syndrome. *Nat Genet* 17:218–222, 1997
50. Wallner EI, Yang Q, Peterson DR, Wada J, Kanwar YS: Relevance of extracellular matrix, its receptors, and cell adhesion molecules in mammalian nephrogenesis. *Am J Physiol* 275:F467–F477, 1998
51. Sbar GD, Venkataseshan VS, Huang Z, Marquet E, Brunswick JW, Churg J: Renal disease in Marfan syndrome. *Am J Nephrol* 16:320–326, 1996
52. Saborio P, Scheinman J: Genetic renal disease. *Curr Opin Pediatr* 10:174–183, 1998